Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

# THE METABOLISM OF CERTAIN RAPIDLY GROWING TUBERCLE BACILLI IN MEDIA WITH INORGANIC SALTS AS SOURCES OF NITROGEN

## STUDIES IN ACID-FAST BACTERIA. IV*

A. I. Kendall, A. A. Day, and A. W. Walker

*(From the Department of Bacteriology, Northwestern University Medical School, Chicago.)*

It is surp to find that an organism as complex in its activities as the tubercl cillus should develop in a medium in which the source of nitrogen is an ammonium salt and the source of carbon an alcohol or a carbohydrate. Wherry[1] has shown that a rapidly growing strain of an avirulent tubercle bacillus will develop, with moderate luxuriance, in a medium even simpler in composition than the asparagin medium, referred to in our Study III. Wherry used ammonium chlorid as a source of nitrogen in his experiments, the various simple alcohols and carbohydrates as sources of carbon. It is essential, as he showed, to add phosphorus to these media, preferably $Na_2HPO_4$, where ammonium chlorid is used as a source of nitrogen, and also a certain amount of NaCl as well. Even avirulent tubercle bacilli do not grow rapidly on this medium, but the growth is unmistakable and it may be increased somewhat in luxuriance by repeated transfers. The morphology of the tubercle bacillus is considerably modified during prolonged growth on this ammonium chlorid medium, the organisms being for the most part non-acid-fast with a preponderance of coccoid elements, some of them containing acid-fast granules, the majority of the granules, however, being metachromatic.

These observations have been repeatedly confirmed in this laboratory. They are of theoretical importance; in the first place, the organisms developing under these conditions may be regarded as tubercle bacilli reduced in their chemical composition to the lowest terms compatible with growth. They contain, theoretically, but seven elements; viz., carbon, nitrogen, hydrogen, oxygen, phosphorus, sodium, and chlorin. It is conceivable that minute traces of sulphur and perhaps

---

* Received for publication July 27, 1914.

[1] Centralbl. f. Bakteriol., Abt. I, Orig., 1913, 70, p. 115; Jour. Infect. Dis., 1913, 13, p. 144.

other elements may be absorbed by the media from the air, but these contaminations, if they were present, were reduced to a minimum by thoroughly cleaning the glassware with which they came in contact with alkalin permanganate, oxalic and chromic acids, and water. The media were made from carefully distilled water. The cultures, furthermore, were protected during incubation by double paper caps, and they were grown in an electrically heated incubator. It would appear, therefore, if these precautions were successful, that living tubercle bacilli, containing but seven elements in their body substance, would develop in a medium containing, as a source of nitrogen, an inorganic salt, ammonium chlorid, and a simple alcohol, ethyl alcohol, as a source of carbon. These organisms, furthermore, can exist indefinitely under these conditions, for cultures in this medium have been carried on several months by repeated transfers without a diminution in the extent of the growth; indeed, there is a tendency for the growth to increase somewhat in luxuriance with repeated transfers.

The development, however, in this medium is slow, and more luxuriant growths are obtainable from the same elements using $(NH_4)_2HPO_4$ as a combined source of phosphorus and nitrogen, the other constituents remaining the same. The metabolism of a rapidly growing tubercle bacillus (597) in a medium consisting of 4 grams of $(NH_4)_2HPO_4$ and 5 grams of NaCl, dissolved in a liter of redistilled water as a basis, to which was added respectively 1 percent of dextrose, 1 percent of mannite, and 3 percent of glycerin as sources of carbon, is presented in this article. The organism was grown for several weeks in this medium, being transferred weekly before the inoculations reported on here were made. It is obvious that this procedure eliminated the possible introduction of small amounts of foreign substances at the time of inoculation and insured successful development of the organism. In experiments involving such simple media, it is obviously essential to exclude extraneous substances by a rigorous attention to the cleaning of all utensils coming in contact with this medium, the details of which have been described. This procedure may be confidently relied on to exclude extraneous contaminations.

It must be remembered that ammonia formation cannot be observed in media in which the source of nitrogen is diammonium hydrogen phosphate, because the ammonia in this compound is removed quantitatively by the Folin air current method in a strongly alkaline solution;[2]

---

[2] Folin, Jour. Biol. Chem., 1912, 11, p. 523.

consequently, the successive determinations of nitrogen in this study are, in reality, determinations of the total nitrogen in solution in the media, and the differences observed between the initial nitrogen content and that found at various stages of growth represent the amounts of nitrogen which is locked up in the bodies of the newly formed bacteria, and perhaps also in some substance or substances formed coincidently by the bacteria which is not detected by this method. If there is such a nitrogen-containing substance or substances, which is not a part of the bacterial cell and which is not detectable by the air current method, it must at least be a synthetic product of bacterial action, for the original nitrogen content can be determined quantitatively by this method.

The tubercle bacillus, according to the analyses of various observers, contains a high percentage of phosphorus amounting to, at least, 50 percent of the ash of the organism,[3] and it is conceivable that some combination of phosphorus with simple amino acids or complex combinations of amino acids might be represented in this non-determinable nitrogen fraction. The experiments of Neuberg and Oertel[4] indicate that the nitrogen of these substances would not escape detection by the air current method, for these substances are labile and easily broken down in acid or alkaline media, and may even decompose on standing.

The results of such a metabolism experiment in this medium are given in Table 1.

The reaction to phenolphthalein in dextrose and mannite is uniformly alkaline; after the first week it becomes progressively acid in glycerin. Inasmuch as the media are fundamentally of the same composition, except for the source of carbon, this experiment would suggest that the organism in question produces acid incidentally to its utilization of glycerin, while it produces alkali when dextrose and mannite are the sources of carbon. This agrees with the observations of Theobald Smith on this point.[5] The reaction in dextrose is slightly acid to alizarin, in mannite it is neutral, but in glycerin it is slightly alkaline. The entire series of observations on changes in reaction to various indicators, when the same organism is grown in media varying in composition from the relatively complex nutrient broth-peptone medium to the simple ammonium phosphate glycerin medium, would suggest that these reactions are due to unknown constituents or com-

---

[3] De Schweinitz and Dorset, Jour. Am. Chem. Soc., 1898, 20, p. 618; 1903, 25, p. 354; and 20th Annual Report of the Bureau of Animal Industry, 1903.

[4] Biochem. Ztschr., 1914, 60, p. 491.

[5] Jour. Med. Research, 1905, 13, p. 253.

## TABLE 1

### METABOLISM OF TUBERCLE BACILLUS IN MEDIA WITH INORGANIC SALTS AS SOURCES OF NITROGEN

| | Dextrose | | | | | Mannite | | | | | Glycerin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days | Alizarin | Neutral Red | Phenol-phthalein | $NH_3$mg. per 100 c.c. Broth | $NH_3$ Total $N_2$ Percent | Alizarin | Neutral Red | Phenol-phthalein | $NH_3$mg. per 100 c.c. Broth | $NH_3$ Total $N_2$ Percent | Alizarin | Neutral Red | Phenol-phthalein | $NH_3$mg. per 100 c.c. Broth | $NH_3$ Total $N_2$ Percent |
| 7 | +0.10 | —0.30 | —0.10 | —3.5 | —8.34 | —0.10 | —0.30 | —0.10 | —3.5 | —8.34 | —0.10 | —0.10 | —0.10 | —4.2 | —10.00 |
| 14 | +0.20 | —0.30 | —0.10 | —4.2 | —10.00 | —0.20 | —0.40 | —0.20 | —4.2 | —10.00 | —0.20 | 0.00 | 0.00 | —4.2 | —10.00 |
| 21 | +0.30 | —0.50 | —0.15 | —4.2 | —10.00 | 0.00 | —0.40 | —0.40 | —4.2 | —10.00 | —0.20 | +0.10 | +0.10 | —4.9 | —11.66 |
| 28 | +0.20 | —0.30 | —0.05 | —2.8 | —6.56 | 0.00 | —0.20 | —0.20 | —2.1 | —5.00 | —0.30 | +0.10 | +0.30 | —4.2 | —10.00 |

binations of constituents of the media, and that they are of value for the identification of these organisms only when the composition of the medium is definitely known, or, in the case of media as complex in composition as the glycerin meat juice peptone broth, where the conditions of preparations are rigorously duplicated each time. This is not to be construed, however, as indicating that these reactions under proper conditions are worthless, for, even in the complex nutrient media ordinarily used for the growth of the tubercle bacillus, these reactions appear to be definite and reliable in the hands of those who pay sufficient attention to the details of composition and preparation. The same mucinous substance, which was found abundantly in cultures of the organism grown in the asparagin medium, was found in the ammonium phosphate medium, altho somewhat less in amount. It was most marked in the mannite medium, less in dextrose, and practically absent in glycerin.

## THE NITROGENOUS METABOLISM IN AMMONIUM PHOSPHATE MEDIUM

The nitrogen content of this medium was uniformly 42 mg. per 100 c.c. At the end of fourteen days the tubercle bacillus (597) had so acted on the nitrogen constituent that 10 percent of it had disappeared from the underlying culture medium; it is probable that a large percentage of this nitrogen was combined in the bodies of the organisms. A certain amount of evidence in favor of this view is afforded by the reappearance of some of this nitrogen in solution in the dextrose and mannite media on the twenty-eighth day. This experiment would suggest that the cause for the recession of the ammonia was, in some way, associated with the autolysis of the pellicle of the tubercle bacilli, which formed on the surface of the medium. There is no recession in the case of the glycerin broth. This observation is in harmony with similar observations in more complex media, where this recession of ammonia was found to be less marked when glycerin was present than when dextrose or mannite were present.

## SUMMARY

A rapidly growing strain of human tubercle bacilli (597) has been grown in a medium of known and very simple composition, consisting essentially of diammonium-hydrogen-phosphate, as a combined source of nitrogen and phosphorus, and dextrose, mannite, and glycerin, respectively, as sources of carbon. At the end of two weeks, 4.2 mg.

of nitrogen, that is to say, 10 percent of the total nitrogen of the uninoculated medium, has been so changed by the growth of this organism that it cannot be recovered as ammonia. This loss of ammonia is most plausibly explained on the assumption that it has been built up into the bodies of the newly developed bacteria. At the end of four weeks, between 40 and 50 percent of this "lost" nitrogen has reappeared in the clear medium underlying the pellicle of the tubercle bacilli in such a form that it can again be determined as ammonia. The period during which the disappearance of nitrogen from the culture fluid is the greatest corresponds with the period of maximum vegetative activity in the culture. Coincidently with the reappearance of this nitrogen, which can be detected as ammonia in solution, there are evidences of a cessation of vegetative activity. This strongly suggests that the reappearance of this ammonia is associated with a certain amount of autolysis of the bodies of the bacteria.